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INFLUENCE OF AUTOCLAVED FUNGAL MATERIALS ON SPEARMINT (*Mentha spicata* L.) GROWTH, MORPHOGENESIS, AND SECONDARY METABOLISM

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Abstract—The influence of autoclaved fungal materials such as culture filtrate, freeze-dried mycelium (FDM), mycelium suspension, and spore suspension (SS) on the growth, morphogenesis, and carvone production of spearmint (*Mentha spicata* L.) plants was studied. Fungal materials were either applied as a drench or spray on the plants. Spearmint plants (cv. "294099^) drenched with SS (1×10^8 spores/ml) of *Trichoderma reesei* showed no significant differences in leaf numbers, root numbers, or shoot numbers compared with nontreated controls. However, significantly higher fresh weights and carvone levels were observed in plants drenched with *T. reesei* SS compared with the untreated controls. Fungal materials derived from *Aspergillus* sp., *Fusarium graminearum*, *F. sporotrichoides*, *Penicillium* sp., *P. acculeatum*, *Rhizopus oryzae*, and *T. reesei* were sprayed on spearmint foliage. *F. graminearum*, *F. sporotrichoides*, or *R. oryzae* elicited no enhanced growth, morphogenesis, or secondary metabolism responses. The best growth and morphogenesis responses were obtained employing *Aspergillus* sp., *Penicillium* sp., or *T. reesei* foliar sprays. For example, spearmint cv. "557807^ plants sprayed with 100 mg/l FDM *T. reesei* isolate NRRL

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[4] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

11460 C30 stimulated higher fresh weights (75%), shoot numbers (39%), leaf numbers (57%), and root numbers (108%) compared with untreated plants. This effect was not dose-dependent because similar growth and morphogenesis responses were obtained by testing 10, 100, or 1000 mg/l FDM concentrations. Carvone levels in fungal-treated foliar-sprayed plants were comparable to nontreated controls. However, total carvone levels per plant were higher in fungal-treated plants because of their increased fresh weight.

Key Words V *Aspergillus*, carvone, freeze-dried mycelium, *Fusarium*, mycelium, *Penicillium*, secondary metabolites, *Rhizopus*, spearmint, spores, *Trichoderma.*

INTRODUCTION

Some biocontrol microorganisms aside from combating pathogens may also cause plants to exhibit enhanced growth (Chang et al., 1986; Ryu et al., 1999, unpublished data; Raj et al., 2003). Microorganism preparations enhancing plant growth may be rhizobacteria (Ryu et al., 1999; Zehnder et al., 2000; Raj et al., 2003) or fungus (Elad, 2000). In these reports, biological preparations were composed of living microorganisms. In contrast, little research has been conducted studying the effects of dead microorganism fractions on growth (i.e., dry or fresh weights) and morphogenesis (i.e., leaf, root, and shoot numbers and sizes) responses in plants. Elad (2000) reported the effective biocontrol of powdery mildew on cucumber (*Cucumis sativus* L.) by application of foliar sprays containing either live or dead *Trichoderma harzianum* T39 cells. In addition, a slight increase in the leaf number occurred in sprayed plants employing either living or dead cells of T39 compared with untreated controls. This study suggested that dead fungal material may elicit both defense and growth and morphogenesis enhancement responses. Effective application of nonliving microorganism fractions to stimulate growth and/or combat pathogens would be of great benefit to the nursery and field grower. The drawbacks of using living microorganisms include their opportunistic human pathogenic nature (e.g., aspergillosis) and the permanent introduction of undesirable foreign fungal populations into soil (Anderson et al., 2002; Barkhage and Bernhard, 2002). These problems would be avoided by employing inert dead fungal materials.

In this study, we sought to determine the influence of applying dead fungal fractions on the growth, morphogenesis, and secondary metabolism responses in spearmint (*Mentha spicata* L.) plants. Secondary metabolites are constitutive chemicals that may represent a first-line defense to pathogen attack (Kuc, 1997). Treatments that stimulate secondary metabolite production could be useful to combat pathogens. The objectives of this study were to evaluate: (1) the effect of drenching the rhizosphere with spore suspensions (rhizosphere stimulation);

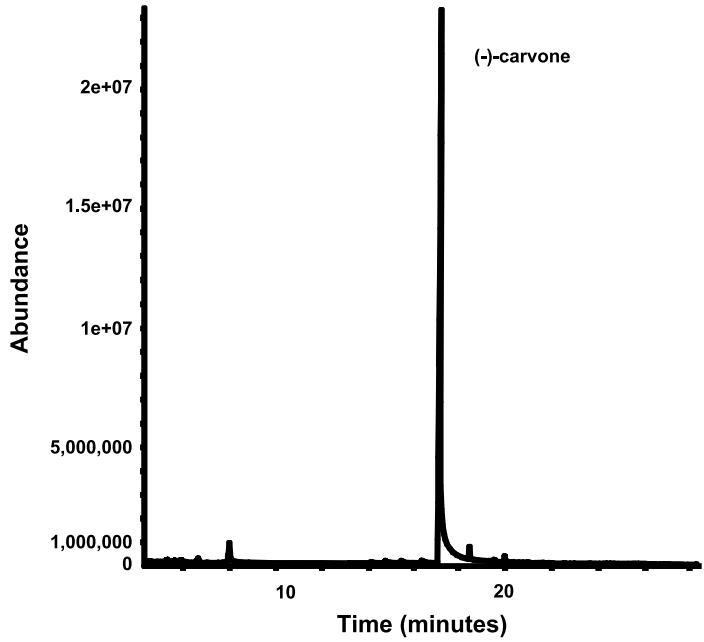


FIG. 1. Typical GC-MS profile of a spearmint plant showing the dominant monoterpene (Y)-carvone in relationship to other monoterpenes present.

(2) the effect of applying suspensions of autoclaved mycelium suspension (MS), freeze-dried mycelium (FDM), spore suspensions (SS), or culture filtrate (CF) as foliar sprays (phylosphere stimulation); and (3) the effect of the concentration of FDM foliar sprays (dosageYresponse stimulation).

Spearmint was employed because it can be easily grown in the greenhouse as uniform-sized rooted shoots that readily manifest growth, morphogenesis, and secondary metabolism responses to various chemical and environmental treatments. The dominant essential oil component in spearmint oil is (−)-carvone, which constitutes >90% of the essential oil composition (Figure 1). Spearmint is commercially grown for its oils, which are employed as fragrance components in toothpastes, mouthwashes, soaps, detergents, lotions, insecticides, and perfumes (Leung, 1980).

METHODS AND MATERIALS

Fungal Cultures and Medium. For the production of SS, fungi isolates (*Aspergillus* sp. NRRL 32534 and *Trichoderma reesei* NRRL 11460 C30) were

grown on potato dextrose agar (PDA) medium at room temperature under fluorescent lighting (12 hr light/12 hr dark) for 7 d. For production of mycelium, fungi isolates (*Aspergillus* sp. NRRL 32534 and NRRL 363, *Fusarium graminearum* NRRL 23639, *F. sporotrichoides* NRRL 3299, *Penicillium* sp. NRRL 32532 and *P. acculeatum* NRRL 2129, *Rhizopus oryzae* NRRL 395, and *T. reesei* NRRL 11460 C30 and NRRL 3652) were grown in liquid potato dextrose broth (PDB) in Fernbach flasks inoculated with fungal cultures maintained on PDA and then placed on a shaker at 250 rpm and incubated at 25°C in the dark. MS were harvested after 72 hr by pouring the mycelium and medium through four layers of sterile cheesecloth followed by squeezing the cheesecloth to extract excess medium and then frozen at −20°C. FDM was prepared by freeze drying MS and then grinding first with mortar and pestle and then with a coffee grinder to produce a fine powder. CFs were collected from liquid PDB and kept in a sterile glass bottle at 0°C until use. All fungal sprays contained 0.025% Tween-80 as a surfactant and were autoclaved at 20 psi at 120°C for 15 min and allowed to cool to room temperature prior to application.

Plant Material. Spearmint stock plants cvs. "294099^ and "557808^ were maintained via shoot cuttings in soil under greenhouse conditions prior to testing with fungal treatments. For experiments, 4-cm tall plants established in Cone-tainers™ (RLC-4 Pine Cell, Stuewe and Sons Inc., Corvallis, OR, USA) containing 10 g of soil were employed. The soil mixture consisted of 1 peat moss/1 vermiculite (v/v) amended with 10.9 g/kg Micromax (Scotts Co., Marysville, OH, USA) and 62.3 g/kg Osmocote 14Y14Y14 (Scotts Co. USA). Plants were grown in a greenhouse at 20 ± 2°C under natural daylight.

Rhizosphere Stimulation. Spearmint (cv. "294099^) plants were given the following drenching treatments (2 ml/cone): water only, 0.1% Tween-80, *Aspergillus* sp. NRRL 32534 SS (1×10^8 spores/ml), *T. reesei* NRRL 11460 C30 SS (1×10^8 spores/ml), 0.5% DMSO, or 1% DMSO. Treatments were administered once a week for two consecutive weeks. Following treatments, plants were grown on greenhouse benches for an additional 4 wk.

Phylosphere Stimulation. The following fungal species and isolates were employed in foliar spray testing: *Aspergillus* sp. NRRL 32534, *F. graminearum* NRRL Z3639, *F. sporotrichoides* NRRL 3299, *Penicillium* sp. NRRL 32532 and NRRL 32533 and *P. acculeatum* NRRL 2129, *R. oryzae* NRRL 395, or *T. reesei* NRRL 11460 C30. Spearmint (cvs. "294099^ or "557808^) plants were given the following fungal foliar sprays: water only, 0.025% Tween-80, 10% PDB, 10% CF, 100 mg/l FDM, 100 mg/l MS, or SS (1×10^8 spores/ml). Plants were sprayed until runoff twice with a 1-wk interval between sprays and then grown on the greenhouse benches for an additional 4 wk.

Dose Response Effect of FDM. FDM suspension from *Aspergillus* sp. NRRL 32534 or NRRL 363, *T. reesei* NRRL 11460 C30 or NRRL 3652, or

Penicillium sp. NRRL 32532 or NRRL 32533 or *P. acculeatum* NRRL 2129 were sprayed on spearmint plants at 10-, 100-, or 1000-mg/l concentrations. Plants were sprayed once a week for 2 consecutive wk and then grown on greenhouse benches for additional 4 wk prior to taking data.

Statistical Analysis. Whole seedlings fresh weight, leaf number per plant, root number per plant, and shoot number per plant were recorded from 5 to 10 plants with the remaining plants employed in essential oil analysis. Experiments were repeated at least twice. Data were analyzed by PC SAS using GLM or ANOVA procedures, and means were separated by Fisher's protected LSD at a 0.05 rejection level.

Essential Oil Analysis. Spearmint plants were randomly selected from each treatment. The top (4 cm from the apical tip) of each individual plant was excised to represent a replicate, and three to five replicates were mixed together per treatment. One gram of fresh plant tissue per treatment was incubated with 15 ml CH_2Cl_2 for 72 hr, allowing carvone levels to come to equilibrium in the solvent. Investigative studies have shown that this method is superior to multiple solvent extractions and subsequent roto-evaporation that result in losses of the volatile carvone (unpublished data). After filtering, samples were analyzed on an HP 5890 gas chromatograph (GC) equipped with a flame ionization detector (FID). GCYmass spectrometry was performed using an HP 6890 Series II gas chromatograph attached to an HP 5972A mass select detector. Columns used were fused silica HP-5MS capillaries (0.25-μm film thickness, 30 ml × 0.25 mm ID). The major compound found in the extracts, (−)-carvone, hereafter referred to simply as carvone, was identified by comparison with mass spectra of a library database, and concentration was calculated from a standard carvone curve. Carvone was measured at mg/g fresh weight.

RESULTS

Rhizosphere Stimulation. Spearmint cv. "294099^ drenched with Tween-80, 0.5% DMSO, or 1.0% DMSO treatments did not show any significant differences for fresh weights, leaf numbers, root numbers, shoot numbers, or carvone levels compared with water-drenched controls. However, spearmint plants drenched with *T. reesei* spores showed higher fresh weights and carvone levels compared with controls, whereas the leaves, shoots, and roots were unchanged compared with controls (Figure 2). Spearmint plants drenched with *Aspergillus* sp. spores showed higher root numbers than control plants, although the other growth and morphogenesis responses were unchanged (Figure 2).

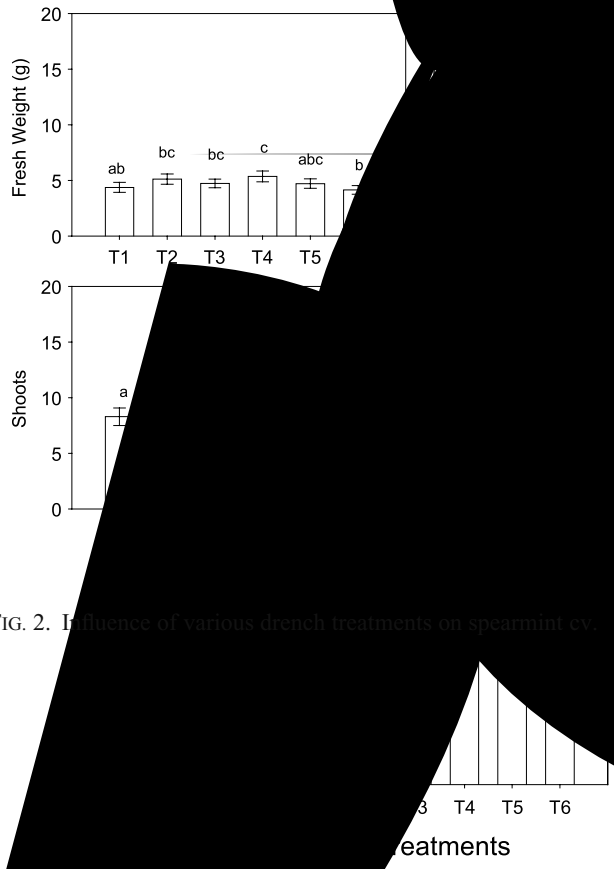


FIG. 2. Influence of various drench treatments on spearmint cv. [illegible]

ation. Plants sprayed with Tween-80, PDB, or DMSO
fference in growth or morphogenesis responses compared
rols (Figures 3Y6). Both spearmint cvs. sprayed with either
T. reesei fungal materials had increased fresh weights, leaf
numbers, and shoot numbers compared with nontreated controls
). For example, spearmint cv. "557808^ plants sprayed with *T.*
RL 11460 C30 FDM or MS increased fresh weights (75 and 121%,
vely), shoot numbers (39 and 70%, respectively), leaf numbers (57 and
, respectively), and root numbers (108 and 108%, respectively) compared

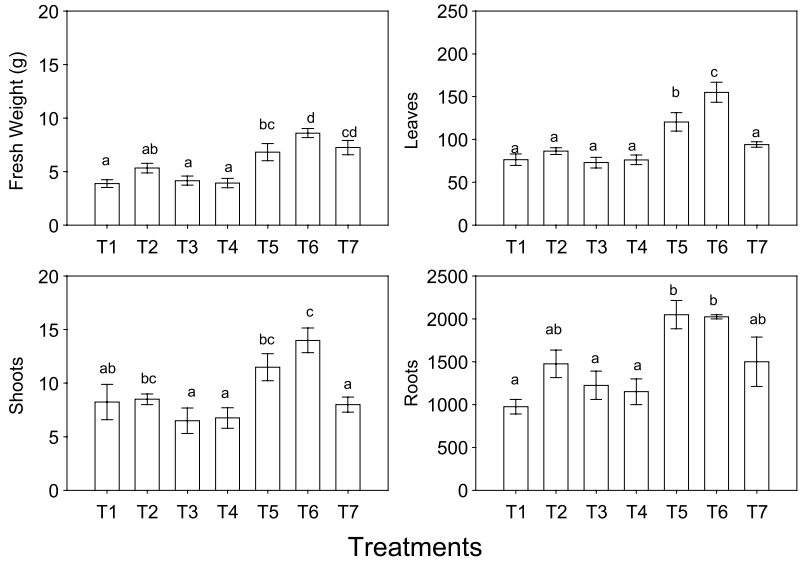


FIG. 3. Influence of *T. reesei* NRRL 11460 C30 foliar sprays on the growth and morphogenesis of spearmint cv. "557807.^ T1 = Control; T2 = Tween-80; T3 = potato dextrose broth; T4 = culture filtrate; T5 = freeze-dried mycelium; T6 = mycelium suspension; and T7 = spore suspension. Columns having the same letters do not differ significantly ($P > 0.05$) in LSD test.

with nontreated controls (Figure 2). In addition, fresh weights were also higher in plants sprayed with SS (87%) compared with controls (Figure 3). Nevertheless, regardless of the fungal material employed, no significant differences were observed among treatments for carvone levels compared with untreated controls ($P > 0.05$, data not shown).

Spearmint cv. "294099^ plants sprayed with *T. reesei* NRRL 32534 CF, FDM, MS, or SS increased fresh weights (36, 53, 47, and 69%, respectively), leaf numbers (84, 91, 56, and 117%, respectively), root numbers (29, 69, 100, and 147%, respectively), and shoots (95, 129, 33, and 67%, respectively) compared with nontreated controls (Figure 4).

Spraying spearmint cv. "557807^ plants with *Aspergillus* sp. NRRL 32534 CF, FDM, MS, or SS increased in fresh weights (88, 81, 108, and 85%, respectively) over nontreated controls (Figure 5). Significant increases in leaf numbers were observed in plants sprayed with FDM, MS, and SS (59, 129, and 69%, respectively) over those of nontreated controls. Significant increases in root numbers in plants sprayed with MS or SS were observed (69 and 69%, respectively) over those of nontreated controls. Plants sprayed with *Aspergillus*

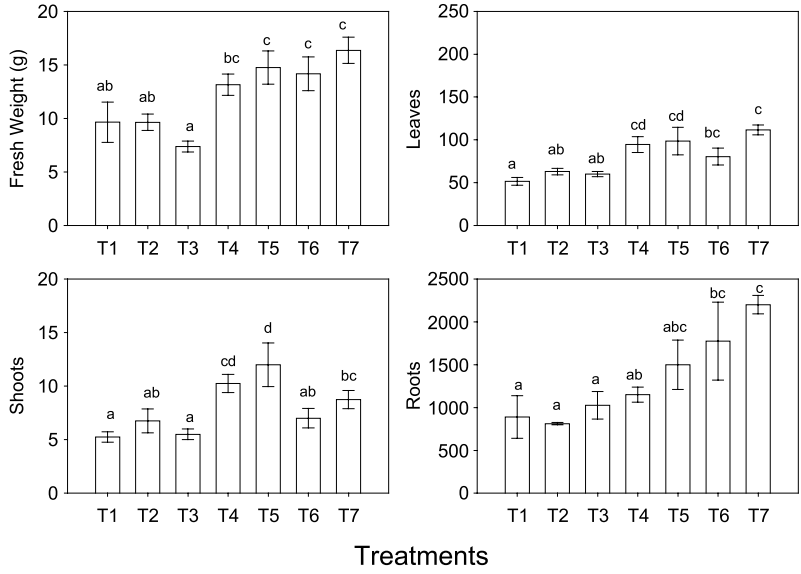
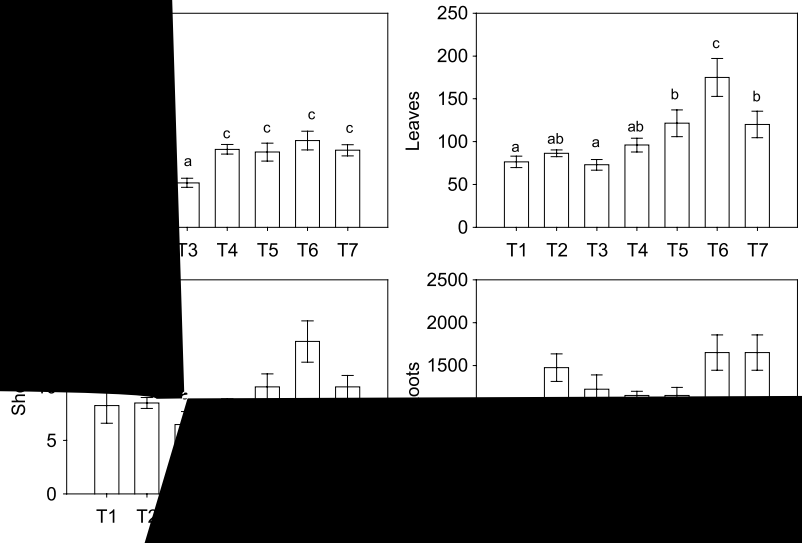


FIG. 4. Influence of *T. reesei* NRRL 11460 C30 foliar sprays on the growth and morphogenesis of spearmint cv. "294099.^ T1 = Control; T2 = Tween-80; T3 = potato dextrose broth; T4 = culture filtrate; T5 = freeze-dried mycelium; T6 = mycelium suspension; and T7 = spore suspension. Columns having the same letters do not differ significantly ($P > 0.05$) in LSD test.

sp. MS also showed significant increases in shoot number (73%) over controls (Figure 5). Spearmint cv. 294099 plants sprayed with *Aspergillus* sp. CF, FDM, MS, or SS caused higher fresh weights (68, 86, 78, and 56%, respectively), leaves (100, 90, 145, and 131%, respectively), roots (139, 55, 98, and 145%, respectively), and shoots (71, 71, 124, and 90%, respectively) compared with those of nontreated controls (Figure 6).

Dosage Response Effect of FDM. Plants sprayed with 10, 100, or 1000 mg/l FDM from isolates of *T. reesei* NRRL 11460 C30 or NRRL 3652 exhibited significant increases in all fresh weights (>41%) and shoot numbers (>54%) (Table 1). Similarly, for all concentrations tested, higher but not always significant increases occurred for root numbers and leaf numbers compared with untreated control plants. No change in carvone levels were observed in any plants treated with any isolate concentration tested (Table 1). Both isolates of *Aspergillus* sp. NRRL 32534 or NRRL 363 administered at 10, 100, and 1000 mg/l caused spearmint plants to exhibit higher (but not always significantly higher) fresh weights, root numbers, shoot numbers, and leaf numbers compared with untreated controls (Table 2). Only spearmint plants treated with *Asper-*



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129 increased
h weights, shoot num
mg/l NRRL 32532),
d root numbers compared with untreated controls (Table 3). Overall, *Peni-*
llium sp. isolates were less effective for the induction of significant increases
n growth and morphogenesis responses compared with other fungal species
isolates tested. Nevertheless, higher and sometimes significant increases in
growth and morphogenesis occurred in plants sprayed with the 100-mg/
l concentrations (except for fresh weights and root numbers with isolate NRRL
32532) compared with that occurring in plants sprayed with the 10-mg/
l concentration regardless of the *Penicillium* sp. isolates tested. Generally,
carvone levels were similar for all isolates, and concentrations tested, except
100 mg/l NRRL 32533, caused a significant increase in carvone production to
occur from treated spearmint plants.

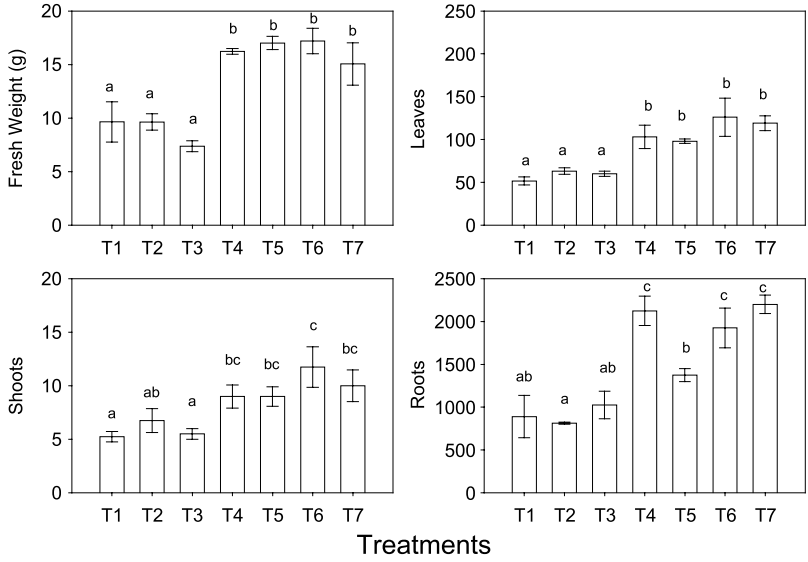

FIG. 6. Influence of *Aspergillus* sp. NRRL 32534 foliar sprays on the growth and morphogenesis of spearmint cv. "294099.^ T1 = Control; T2 = Tween-80; T3 = potato dextrose broth; T4 = culture filtrate; T5 = freeze-dried mycelium; T6 = mycelium suspension; and T7 = spore suspension. Columns having the same letters do not differ significantly ($P > 0.05$) in LSD test.

DISCUSSION

In this study, we have identified isolates of dead fungi that enhance spearmint growth, morphogenesis, and secondary metabolism. Aside from the enhanced leaf production noted by Elad (2000), the application of dead fungal cells to plants to enhance growth has not been reported in the literature. Drenching soil with fungal spores was found to be a less effective method of promoting growth and morphogenesis responses on spearmint plants than foliar sprays. For some fungal species (i.e., *F. graminearum*, *F. sporotrichoides*, and *R. oryzae*), foliar sprays failed to elicit any response whatsoever in terms of growth, morphogenesis, or secondary metabolism in spearmint, whereas other fungal species foliar sprays such as from *Aspergillus* sp. and *T. reesei* elicited strong growth and morphogenesis responses. Clearly, the species and isolate type appear important in obtaining growth and morphogenesis responses. The method of application (foliar spray vs. drench) and type of fungal material (CF, FDM, MS, or SS) employed are critical to obtaining increased growth and morphogenesis responses. We also found that growth and morphogenesis were

TABLE 1. EFFECT OF FOLIAR SPRAYS OF *T. reesei* NRRL 11460 C30 OR NRRL 3652 ON THE GROWTH, MORPHOGENESIS AND SECONDARY METABOLISM OF SPEARMINT CV. "557807^

	Percentage increase over control/mean ± standard error				
Treatments[a]	Root number	Shoot number	Leaf number	Fresh weight (g)	Carvone (mg/g fwt)
T1	0	0	0	0	0
	1650.0 ± 125.83	6.5 ± 0.65	112.25 ± 8.17	9.66 ± 0.92	2.34 ± 0.12
T2	37.88	12.0	3.79	9.97	−16.7
	2275.0 ± 103.1	7.25 ± 0.75	116.5 ± 6.6	10.62 ± 0.91	1.95 ± 0.08
T3	42.42	88.46	29.18	54.17	−11.5
	2350.0 ± 236.29	12.25 ± 1.49	145.0 ± 11.1	14.89 ± 0.88	2.07 ± 0.1
T4	30.3	88.46	37.42	58.28	18.5
	2150.0 ± 95.74	12.25 ± 0.85	154.25 ± 8.37	15.29 ± 0.62	2.77 ± 0.14
T5	45.45	53.85	16.04	50.56	−7.3
	2400.0 ± 158.1	10.0 ± 1.41	130.25 ± 10.28	14.54 ± 1.07	2.17 ± 0.09
T6	62.12	119.24	68.82	58.07	−2.1
	2675.0 ± 137.69	14.25 ± 0.75	189.5 ± 14.97	15.27 ± 1.76	2.29 ± 0.27
T7	36.36	80.77	38.08	41.19	0.9
	2250.0 ± 221.74	11.75 ± 1.38	155.0 ± 17.87	13.64 ± 0.57	2.36 ± 0.15
T8	54.54	57.69	24.28	45.14	−8.5
	2550.0 ± 275.38	10.25 ± 0.75	139.5 ± 11.29	14.02 ± 0.8	2.14 ± 0.26
LSD[b]	526.61	3.1	33.92	2.93	0.5

[a]T1 = Control; T2 = Tween-80; T3 = 10 mg/l NRRL 11460 C30; T4 = 100 mg/l NRRL 11460 C30; T5 = 1000 mg/l NRRL 11460 C30; T6 = 10 mg/l NRRL 3652; T7 = 100 mg/l NRRL 3652; T8 = 1000 mg/l NRRL 3652.
[b]Means were separated by Fisher's protected LSD test ($P > 0.05$).

enhanced more by FDM, MS, or SS than by using CF. We usually did not observe any differences between employing FDM, MS, or SS on the growth and morphogenesis responses obtained (Figures 3Ÿ6). CF activity was inconsistent and may or may not enhance growth and morphogenesis in spearmint depending on the isolate employed. Both clones of spearmint reacted similarly in the presence of fungal isolates, types, and concentrations. Our results confirm those by Elad (2000) in which leaf number in cucumber was enhanced by the application of dead *T. harzianum* T39 cells. In addition, we noted strong positive correlations between fresh weight, shoot number, leaf number, and root number responses when growth or morphogenesis is stimulated in the presence of dead fungal materials.

We did not promote any significant increases in absolute mint carvone levels by using any of the fungal foliar spray studies. Apparently, enhanced growth and morphogenesis is not associated with enhanced secondary metabolism. However, inert dead fungal material applications did not reduce

TABLE 2. EFFECT OF FOLIAR SPRAY OF *Aspergillus* sp. NRRL 32534 OR NRRL 363 ON GROWTH, MORPHOGENESIS AND SECONDARY METABOLISM OF SPEARMINT CV. "557807^

Treatments[a]	Percentage increase over control/mean ± standard error				
	Root number	Shoot number	Leaf number	Fresh weight (g)	Carvone (mg/g fwt)
T1	0	0	0	0	0
	1650.0 ± 125.83	6.5 ± 0.65	112.25 ± 8.17	9.66 ± 0.92	2.34 ± 0.12
T2	37.88	11.54	3.78	9.97	−16.7
	2275.0 ± 103.1	7.25 ± 0.75	116.5 ± 6.6	10.62 ± 0.91	1.95 ± 0.08
T3	60.6	76.92	4.09	16.63	−22.2
	2650.0 ± 347.61	11.5 ± 3.33	157.25 ± 32.25	11.27 ± 0.52	1.82 ± 0.19
T4	57.58	115.38	71.05	34.42	−15.0
	2600.0 ± 70.71	14.0 ± 1.47	192.0 ± 23.71	12.98 ± 1.59	1.99 ± 0.14
T5	59.09	130.77	58.13	50.68	−9.0
	2625.0 ± 278.01	15.0 ± 2.04	177.5 ± 25.17	14.55 ± 2.1	2.13 ± 0.15
T6	16.67	69.23	38.31	29.4	12.4
	1925.0 ± 188.75	11.0 ± 0.71	155.25 ± 8.4	12.5 ± 0.68	2.63 ± 0.18
T7	56.06	57.69	42.98	44.97	−6.0
	2575.0 ± 356.78	10.25 ± 0.85	160.5 ± 21.88	14.0 ± 1.45	2.2 ± 0.24
T8	24.24	80.77	33.63	23.03	5.6
	2050.0 ± 95.74	11.75 ± 0.95	150.0 ± 6.68	11.88 ± 0.9	2.47 ± 0.07
LSD[b]	654.02	4.67	55.96	3.61	0.47

[a]T1 = Control; T2 = Tween-80; T3 = 10 mg/l NRRL 32534; T4 = 100 mg/l NRRL 32534; T5 = 1000 mg/l NRRL 32534; T6 = 10 mg/l NRRL 363; T7 = 100 mg/l NRRL 363; T8 = 1000 mg/l NRRL 363.

[b]Means were separated by Fisher's protected LSD test ($P > 0.05$).

carvone levels compared with untreated control plants. Secondary metabolites have been found to increase in plants in response to microorganism infection and stress (Bell, 1981; Cheniclet et al., 1988; Dalkin et al., 1990; Dixon and Lamb, 1990). For example, terpene content increased when maritime pine (*Pinus pinaster*) was attacked by *Tomicus piniperda* beetle (Cheniclet et al., 1988). Inclusion of dead microorganisms into nutrient medium is commonly employed to enhance secondary metabolite production in plant cell suspension cultures (Heinstein, 1985; Eilert and Contabel, 1986; Eilert et al., 1986; Dalkin et al., 1990; Mahady and Beecher, 1994). For example, Mahady and Beecher (1994) reported that addition of *Penicillium expansum* to a culture of *Sanguinaria canadensis* induced the production of benzophenanthridine alkaloids such as sanguinarine and chelerythrine in a dose-dependent manner. Similarly, the addition of fungal elicitors to cultures of *Papaver somniferum* stimulated sanguinarine production (Eilert et al., 1986). It is interesting that these fungal additions were administered typically at the end of the cell

TABLE 3. EFFECT OF FOLIAR SPRAY OF *Penicillium* sp NRRL 3232 AND NRRL 32533 OR *P. acculeatum* NRRL 2129 ON GROWTH, MORPHOGENESIS AND SECONDARY METABOLISM OF SPEARMINT CV. "557807^

	Percentage increase over control/mean ± standard error				
Treatments[a]	Root number	Shoot number	Leaf number	Fresh weight (g)	Carvone (mg/g fwt)
T1	0	0	0	0	0
	1650.0 ± 125.83	6.5 ± 0.65	112.25 ± 8.17	9.66 ± 0.92	2.34 ± 0.12
T2	37.88	11.54	3.79	9.97	–16.7
	2275.0 ± 103.1	7.25 ± 0.75	116.5 ± 6.6	10.62 ± 0.91	1.95 ± 0.08
T3	54.55	30.77	0	46.12	3.0
	2550.0 ± 165.83	8.5 ± 1.26	111.0 ± 5.12	14.11 ± 1.02	2.41 ± 0.24
T4	31.82	73.08	24.94	44.42	–28.2
	2175.0 ± 118.15	11.25 ± 2.36	140.25 ± 20.95	13.95 ± 1.24	1.68 ± 0.16
T5	18.18	26.92	7.35	15.63	25.6
	1950.0 ± 210.16	8.25 ± 1.31	120.5 ± 10.96	11.17 ± 0.55	2.94 ± 0.09
T6	21.21	103.85	41.87	34.39	7.3
	2000.0 ± 204.12	12.98 ± 2.19	159.25 ± 4.42	12.98 ± 2.19	2.51 ± 0.08
T7	18.18	26.92	21.38	18.49	–13.2
	1950.0 ± 50.0	8.25 ± 0.75	136.25 ± 6.56	11.44 ± 0. 7	2.03 ± 0.12
T8	66.67	69.23	28.51	21.38	–17.5
	2550.0 ± 275.38	11.0 ± 1.08	144.25 ± 5.14	11.72 ± 0.78	1.93 ± 0.08
LSD[b]	434.26	3.55	28.9	3.34	0.4

[a]T1 = Control; T2 = Tween-80; T3= 10 mg/l NRRL 32532; T4 = 100 mg/l NRRL 32532; T5 = 10 mg/l NRRL 32533; T6 = 100 mg/l NRRL 32533; T7 = 10 mg/l NRRL 2129; T8 = 100 mg/l NRRL 2129.

[b]Means were separated by Fisher's protected LSD test ($P > 0.05$).

suspension growth cycle. It is not reported if the fungal additives stimulated or depressed cell suspension growth.

Under favorable environmental conditions, plants prioritize their resources towards growth and differentiation over secondary metabolism (Bazzaz et al., 1987; Chapin et al., 1990; Dickson and Isebrands, 1991). Plant growth processes require high amounts of primary carbon resources that may come at the cost of secondary metabolite production (Gulmon and Mooney, 1986; Williams et al., 1987; Margna et al., 1989; Lambers and Rychter, 1990; Chapin, 1991). Generally, in our study, there was no significant change in carvone levels in spearmint plants that showed significant increases in growth and morphogenesis over untreated controls. However, because of the increases in growth, the total carvone levels per plant were significantly higher in fungal-treated plants than untreated spearmint plants.

Dead fungal cell stimulation of plant growth and differentiation may be either chemical or physical or both. If the phenomenon is physical, the plants

may perceive themselves under attack by fungal cells and in response initiate rapid growth. If chemical, we speculate that dead fungal cells may emit a stimulatory chemical substance that then encourages plant growth.

Currently, we are extending our studies into this phenomenon by examining various fungal species and isolates with different plant species. Thus far, the results confirm those presented in this paper. These studies may lead to industrial products that can be used as growth stimulators for a variety of nursery and greenhouse plants. For two of the fungi (*T. reesei* and *Aspergillus* sp.) examined in this study, both are employed in the paper pulp processing (Toyama et al., 2002) and enzyme production (Chen and Reese, 2002) industries. Perhaps mycelium suspension, which is currently obtained as a waste product, may have potential as a plant growth promoter.

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